A Quantitative Assessment of the Microbiological Quality of Lebanese Tahini (Sesame Paste)

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***Abstract*-The microbiological quality of tahini produced by several manufacturers in Lebanon was evaluated. Sixty-three tahini samples were collected randomly from retail markets throughout the country with production dates ranging from October 2015 to September 2017. The majority of the samples were from companies that are international exporters of the product. Nine of the obtained samples were from a traditional tahini manufacturer. All samples were assessed for the total Aerobic Plate Count, the presence and enumeration of *Staphylococcus aureus*, yeasts and molds, *Salmonella*, coliforms and *Escherichia coli*. Spread plate methods were used for detection and enumeration. The following results were obtained: the Aerobic Plate Count of the samples ranged between $1x10^2$ CFU/g and $8.2x10^5$ CFU/g with an average of $8.2x10^4$ CFU/g. *S. aureus* counts ranged between <20 CFU/g and $9.2x10^3$ CFU/g with an average of $8.3x10^2$ CFU/g. Yeasts and molds were present at counts ranging from <10 CFU/g to $2.2x10^5$ CFU/g with an average of $2.5x10^4$ CFU/g. Total coliform counts ranged between <30 CFU/g and $3.4x10^5$ CFU/g with an average of $2.3x10^4$ CFU/g. *E. coli* was present in ~37% of the samples (23 out of 63), while *Salmonella* was confirmed present in ~16% of the samples (10 out of 63). When compared with local and international standards, many of the samples showed unacceptable levels of microbial contamination. Certain impact factors were also determined when the samples were grouped according to their respective manufacturer, age, and processing method.**

Keywords-Tahini; Sesame Paste; Microbiology; Lebanon.

I. INTRODUCTION

Tahini is a well-known Middle Eastern condiment made from toasted ground hulled sesame seeds [1]. The paste has gained popularity all over the globe as a result of its health and culinary benefits [2]. In 2014, the Middle East and Mediterranean tahini market was estimated to be at a value of $783.9 Million, with forecasts of a further escalation by 2020. Lebanon has been an important exporter of tahini, and is home to many key players in the market [3].

The importance of tahini comes from the fact that it is used commercially and at a household level as an ingredient in many cultural delicacies. These include products that have gained international popularity, such as hummus (chickpeas with tahini), and mtabal betejen (roasted eggplant and tahini) [4]. The paste is also used as a sauce for meats like *shawarma*, and as a sauce (known as tarator) for fish and *falafel*. Tahini also makes up about 50% of halva (or halawa), a sweet made up of tahini, sugar, citric acid and *Saponaria officinalis* root extract [5]. Tahini is of high nutritive value. It is rich in lipids, proteins, carbohydrates, niacin, thiamin, and some minerals like calcium, and phosphorous [6]. The traditional way of tahini processing in Lebanon includes: sorting the seeds to remove dark or imperfect seeds, followed by soaking the seeds in salt water. This helps settle impurities and dirt at the bottom and ease the peeling process. The seeds that are floating on the surface of the water are then collected, peeled and washed. The next step involves roasting the seeds, followed by the stone-grinding phase, which brings out the oil in the sesame and turns it into a paste. Many tahini manufacturers, however, rely on a fully automated process. Instead of soaking the seeds in salt water, they are passed into a centrifuge that separates any impurities. The sesame then enters a washing machine, followed by a drying machine and then a roaster. The roasted sesame is cleaned once again and sorted by color. The accepted seeds then undergo grinding, are homogenized and then finally pasteurized at a high temperature for several hours to get rid of any potential bacteria [7][8] (Figure 1).

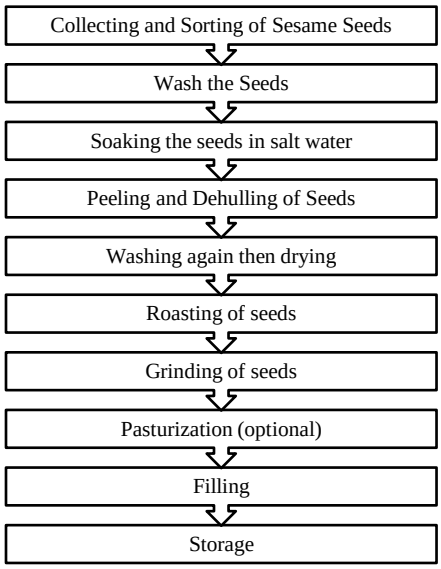


Figure 1. Schematic diagram showing the basic steps for tahini processing from sesame seeds.

After production tahini is stored at room temperature and has a shelf life up to 2 years [9]. It is typically consumed directly and does not require any further processing. Therefore, it should be free from any pathogenic bacteria upon packaging. The raw sesame itself should also be free from microbes, so as not to increase the risk of contamination [10]. However, despite the development of a hazard analysis critical control point (HACCP) plan for the manufacturing of tahini [11], in recent years, sesame paste has emerged as a product of concern, with many of the end products containing *Salmonella*, *Staphylococcus aureus*, *Escherichia coli*, and a number of other hazardous microbes. In addition tahini has a low water activity (~0.16) as well as low pH (~5.9) [11], conditions that permit the growth of many foodborne microorganisms [12].

The presence of microbes has been attributed to a number of reasons including, the microbial quality of sesame seeds, poor hygiene and sanitation, and improper processing and storage conditions [10]. Outbreaks of *Salmonella* infections have been traced back to tahini, some particularly correlated with Lebanese products [13]. Though some studies have dealt with the microbiological quality of sesame seed products, a collective investigation into tahini products in Lebanon using conventional plating methods has yet to be established. Therefore, the objective of this study will be to detect and enumerate microbial contamination of tahini in Lebanon, while also checking for possible impact factors including the processing method, storage time, or difference among products due to difference in manufacturer.

This paper includes four sections. Aside from the introduction, Section II includes a detailed description of the materials and methods used in the study. In Section III we mention the microbial results obtained and whether grouping the samples according to their corresponding manufacturer, method of processing, or sample age may have had an impact on the obtained results, which we discuss in accordance to similar studies. In the final section, Section IV, we wrap up our research in a concluding statement and mention some limitations, as well as possible future work in the related area.

II. MATERIALS AND METHODS

A. Sampling

Sixty-three tahini samples with production dates varying from October 2015 to September 2017 were collected from retailers and producers throughout certain areas in Lebanon. Nine of the samples were obtained from a traditional manufacturer (no automated machinery). Sample weights varied between 200g and 900g. All samples were held at room temperature (25^0C) and collected in their original packages, which were wiped with ethanol before testing. The samples were given a letter based on the manufacturer as shown in Table I. Using a sterilized rod, the samples were thoroughly mixed. 25 g of each sample were then transferred aseptically into separate sterile plastic bags containing 225 ml of buffered peptone water for homogenization. Homogenization was carried out using a stomacher (Model, 1605 BL Smart) for 2 minutes. Following homogenization ten-fold serial dilutions up to 10^3 were prepared, and inoculated on appropriate media.

B. Microbial Analysis

Aerobic Plate Counts (APC), *Staphylococcus aureus*, coliforms, and yeasts/mold counts were determined for each sample, as well as the presence or absence of *Escherichia coli*, and *Salmonella.*

Aerobic Plate Count. APC was determined according to the procedure specified by Morton R.D [14]. 0.1 ml of each dilution was inoculated and spread onto Plate Count Agar (PCA) (HiMedia) and left to dry. The plates were then incubated at 35 ± 1^0C for 48 ± 2 hours.

Staphylococcus aureus. *S. aureus* was detected and enumerated via surface plating 0.5 ml on Mannitol Salt Agar (MSA) (HiMedia) and incubating plates at 35 ± 1^0C for 48 ±2 hours. Colonies with typical and atypical *S. aureus* morphology were confirmed by the catalase and coagulase tests. This method is in accordance with that specified by the British Standards Institution, with a modification of the agar [15].

Yeast and Mold. Yeast and mold counts were determined following spread plate inoculation onto Saubaurad Dextrose Agar (SDA) (HiMedia). Plates were incubated at 25 ± 1^0C for 5 days. This procedure was taken from the United States Food and Drug administration (FDA) [16] however the proposed agar was substituted with SDA.

Total coliforms and Escherichia coli. Total coliforms were enumerated on Eosin Methylene Blue Agar (EMB) (HiMedia) [17]. An addition to the procedure determined by Gehm & Heukelekian included pre-enrichment of 1 ml of the samples with 10 ml Lactose Broth (HiMedia) for 48 hours, at an incubation temp of 35 ± 1^0C. Following the pre-enrichment step, 1 ml of each dilution was surface plated onto EMB agar plates and incubated at 35 ± 1^0C for 48 ±2 hours. Plates with typical *E. coli* colonies were confirmed for presence of the bacteria via biochemical IMViC tests (HiMedia).

Salmonella. For detecting *Salmonella*, the FDA Bacteriological Analytical Manual (BAM) procedure was implemented, with some modifications [18]. Pre-enrichment was carried out by suspending 25g of each sample in 225ml of Lactose Broth (HiMedia), followed by incubation at 35 ± 1^0C for 24±2 hours. 1 ml of each sample was then transferred to 10 ml tubes of Selenite F Broth (SFB) (HiMedia) and incubated at 35 ± 1^0C for 24±2 hours. After incubation, 3 mm loopfuls were streaked onto *Salmonella* Shigella agar (SS)(HiMedia) and incubated for another 24 ±2 hours. Typical and atypical colonies for presumptive *Salmonella* were then transferred to Kliger Iron Agar (KIA) (HiMedia). Confirmation was carried out via IMViC biochemical tests (HiMedia), Urea Broth (HiMedia), and Phenol D broth (HiMedia).

C. Statistical analysis

The data was analyzed using analysis of variance (ANOVA) completely randomized design. Differences

among means of the treatments were analyzed using Duncan. Significant differences were determined when $p \leq 0.05$. Significant differences for means obtained after grouping the samples based on the processing method, were determined using independent t-test analysis.

III. Results and Discussion

A. Microbial Counts

APC, *S. aureus*, total coliform, and yeast and mold counts, as well as the presence or absence of *Salmonella* and *E. coli* obtained following assessment of sixty-three tahini samples in Lebanon are shown in Table I. APCs for the samples ranged between $1x10^2$ colony forming unit per gram (CFU/g) and $8.2x10^5$ CFU/g with an average of $8.2x10^4$ CFU/g. *S. aureus* counts ranged between <20 CFU/g and $9.2x10^3$ CFU/g with an average of $8.3x10^2$ CFU/g. Yeasts and molds were present at counts ranging from <10 CFU/g to $2.2x10^5$ CFU/g with an average of $2.5x10^4$ CFU/g. Total coliform counts ranged between <30 CFU/g and $3.4x10^5$ CFU/g with an average of $2.3x10^4$ CFU/g. *E. coli* was present in 36.5% of the samples (23 out of 63), while *Salmonella* was confirmed present in 15.9% of the samples (10 out of 63).

Similar studies have been done on the microbial quality of tahini and similar results were obtained. A similar study in Saudi Arabia, revealed APC levels for 50 tahini samples at an average of $2x10^4$ CFU/g, slightly lower than the obtained average of the current study [19]. Another study on tahini samples, assessed directly after manufacturing and 4 months after production, was done in Jordan, and the highest average for APC of $5.3x10^3$ CFU/g was still lower than the obtained average [11]. Al-Sogair et al. (1986) revealed lower averages for *S. aureus* at 56 CFU/g and much lower levels of yeast and mold, ranging from <10 to 50 CFU/g [19]. Yamani & Isa (2006) determined *S. aureus* levels to also be at 54 CFU/g respectively while the average for yeast and mold counts was $1x10^2$ CFU/g [11]. The current average value for total coliform counts was $2.3x10^4$ CFU/g, also a high average compared to average counts of 49 CFU/g, and $6x10^2$ CFU/g for comparable studies [19][11].

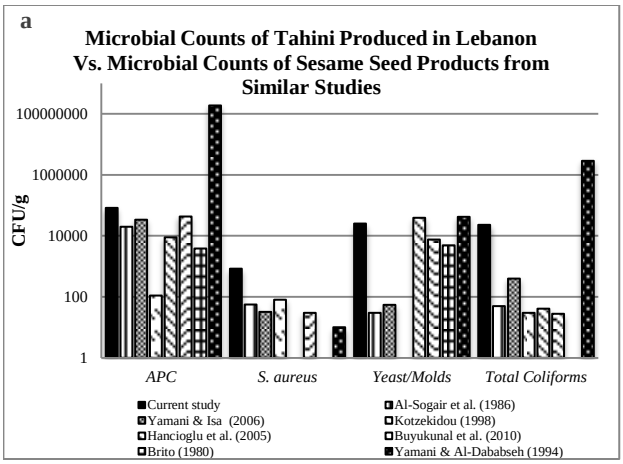
This is not the first time *Salmonella* is detected in tahini. The microbial assessment of tahini samples on the shelves of retail markets has led to the recall of some products [20][13]. Al-Sogair et al. (1986), in a similar study, detected *Salmonella* in 20% of the examined samples as well [19].

The microbial quality of sesame paste products, such as halva and hummus, have also been investigated. Figure 2 compares microbial counts obtained from some literature with those obtained from Lebanese manufactured tahini associated with this study, and shows somewhat similar results. However, *S. aureus* levels were the highest in tahini manufactured in Lebanon, while APC and total coliform counts were also the second highest compared to other literature. Comparing these values to standards of acceptance will determine just how much of a health hazard Lebanese tahini is.

TABLE I. Labenese Tahini Microbial Analysis Results

		Microbial Quality CFU/g[a]				
Manufacturer	***APC***	***S. aureus***	***Yeast/Molds***	***Total coliforms***	***E. coli***	***Salmonella***
A	$3x10^2$	60	$4x10^2$	$1.4x10^4$	+	-
B	$5x10^2$	60	$1x10^2$	$2.2x10^2$	-	-
C	$4x10^2$	60	$1x10^2$	$3.7x10^3$	+	+
D	$6.8x10^2$	<20	<10	$7.30x10^3$	+	-
E	$2.3x10^3$	$2.2x10^2$	<10	$2.1x10^3$	-	+
F	$7x10^2$	60	$6x10^2$	>300	-	+
G	$8.8x10^4$	$3.8x10^2$	3x103	$3x10^4$	+	-
A	$1x10^2$	$2x10^2$	<10	<30	-	+
B	$1.2x10^4$	$1.8x10^2$	$6.2x10^3$	$2.5x10^4$	+	-
C	$4.5x10^4$	$9.2x10^3$	$4.4x10^4$	$4.8x10^3$	+	-
D	$3x10^2$	60	$4x10^4$	$6.6x10^3$	-	-
E	$6x10^2$	<20	$7x10^2$	$7x10^4$	+	-
F	$1x10^3$	$3.2x10^2$	$1x10^2$	<30	-	-
G	$3x10^2$	40	$4x10^2$	$2x10^3$	+	-
A	$7.5x10^3$	$2.8x10^2$	$1.5x10^3$	$2.5x10^3$	-	-
B	$1.4x10^3$	$1.3x10^2$	$1.9x10^3$	$4x10^3$	-	-
C	$3.2x10^3$	$2x10^2$	$8.3x10^3$	$2.3x10^4$	-	+
D	$6.3x10^3$	50	$3x10^2$	<30	-	-
E	$2.5x10^3$	$1.1x10^2$	<10	$5x10^2$	-	-
F	$2x10^2$	<20	<10	$2.6x10^3$	+	-
G	$1.2x10^4$	60	$1.2x10^4$	$1.2x10^4$	-	-
A	$7x10^2$	60	$2.3x10^3$	$1.3x10^4$	+	-
B	$1x10^2$	<20	$1.5x10^4$	$1x10^2$	-	-
C	$4x10^4$	$1.2x10^2$	$1.5x10^5$	$4x10^2$	+	-
D	$3.1x10^5$	$1.2x10^2$	$7x10^2$	$2.9x10^4$	-	-
E	$2.5x10^5$	<20	$1x10^3$	$1.6x10^4$	+	+
F	$6.5x10^4$	$5.6x10^3$	$1.2x10^5$	$4.3x10^3$	+	-
G	$2.3x10^4$	<20	$5.2x10^4$	$4.1x10^3$	+	-
A	$5x10^4$	40	$1.8x10^4$	$1x10^2$	-	-
B	$1.5x10^4$	$2.2x10^2$	$7.8x10^4$	$2x10^4$	-	+
C	$2.3x10^5$	$1.6x10^2$	$3.8x10^4$	$4x10^4$	+	-
D	$5x10^2$	<20	$6.2x10^3$	$2.2x10^3$	-	-
E	$6x10^3$	80	$3x10^3$	$3.5x10^4$	-	-
F	$2.2x10^3$	$3x10^2$	$1.2x10^4$	$2.2x10^4$	-	-
G	$2x10^2$	<20	$1.9x10^4$	$1.5x10^3$	+	-
A	$5.5x10^4$	$3.2x10^2$	$3.4x10^4$	$3.2x10^5$	-	-
B	$6.2x10^5$	$1x10^2$	$1.2x10^5$	$2.2x10^5$	-	-
C	$3.3x10^5$	$5.4x10^2$	$1.1x10^5$	$3.4x10^5$	-	-
D	$3.3x10^5$	80	$6.4x10^3$	$1.2x10^5$	+	-
E	$1.5x10^3$	40	$1.1x10^5$	$9.3x10^4$	-	-
F	$1.1x10^5$	$2.2x10^2$	$8.4x10^3$	$5.2x10^3$	+	-
G	$1.3x10^4$	<20	$3.2x10^2$	$1x10^2$	-	-
A	$1.1x10^3$	$6.8x10^2$	$7.8x10^2$	$2.6x10^3$	+	-
B	$1.4x10^3$	$5x10^2$	$1.4x10^4$	$1.8x10^3$	-	-
C	$2.4x10^3$	$3.6x10^2$	$8.2x10^4$	$1.9x10^4$	-	-
D	$1.7x10^4$	$3.4x10^2$	$5.4x10^3$	$3.6x10^2$	+	-
E	$1.5x10^4$	80	$3x10^3$	$5.6x10^2$	-	+
F	$1.2x10^5$	$5x10^3$	$7.6x10^4$	$1.2x10^3$	+	-
G	$5.8x10^3$	<20	$9x10^2$	<30	-	-
A	$3.3x10^3$	$1.4x10^3$	$4.5x10^4$	$2x10^2$	-	-
B	$1x10^4$	$1.2x10^3$	$6x10^2$	<30	-	+
C	$9.6x10^3$	$9.2x10^3$	$2.1x10^2$	$6.3x10^2$	-	-
D	$2.4x10^4$	$3.6x10^2$	$7x10^2$	$2.3x10^3$	+	-
E	$8.3x10^3$	$8.8x10^2$	$3.3x10^4$	$9.2x10^2$	-	-
F	$5.3x10^5$	<20	$2.4x10^3$	$1.6x10^2$	-	-
G	$1.5x10^3$	<20	900	<30	-	-
A	$5.3x10^4$	$2.4x10^2$	$8.2x10^3$	$5.2x10^2$	-	-
B	$3.2x10^5$	40	$5.6x10^4$	60	-	-
C	$2.5x10^5$	$6.6x10^3$	$2.2x10^5$	$2.4x10^4$	-	-
D	$2x10^4$	$8.4x10^2$	<10	<30	-	-
E	$8.2x10^5$	$4.8x10^3$	$1.5x10^3$	$5.3x10^2$	+	+
F	$8.3x10^3$	80	$2.5x10^4$	$1.3x10^4$	-	-
G	$3.4x10^4$	80	$2.7x10^4$	$3.3x10^2$	-	-
Average	**$8.2x10^4$**	**$8.3x10^2$**	**$2.5x10^4$**	**$2.3x10^4$**	**36.5%**	**15.9%**

a Average of duplicate replications, CFU/g=Colony forming unit per gram, Note: + unacceptable microbe quantities, - absence of microbe/ present but in acceptable amount



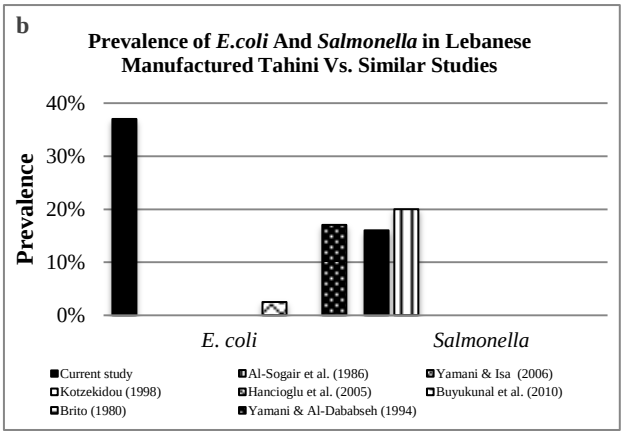


Figure 2. Microbial counts of tahini produced in Lebanon vs. Microbial counts of sesame seed products from similar studies. (a) APC, *S. aureus*, Yeast/mold, total coliform counts. (b) Prevalence of *E. coli* and *Salmonella*, CFU/g= Colony forming unit per gram, References [11], [19], [21],[22], [23], [24], [25] were used.

B. *Comparing Microbial Levels to Local and International Standards*

Lebanese standards set the maximum limit for APCs, yeast and molds, *E. coli* and *Salmonella* at $1x10^4$ CFU/g, $1x10^3$ CFU/g, 10 CFU/g, and 0 CFU/g respectively, beyond which microbial content could prove hazardous upon consumption [26]. As seen in Table II, a considerable amount of the samples analyzed contained unacceptable microbial content. Almost half of the samples, (46%) contained unacceptable quantities of APC, while more than half of the samples showed unacceptable quantities of *S. aureus* (52%), yeast and mold quantities (67%), and coliform counts (83%). *E. coli* was detected in 37% of the samples. Even minute amounts of *Salmonella* are detrimental to one's health and therefore 16% of the samples were found to be hazardous.

Standards available from the Gulf countries set the limit for APCs, *S. aureus*, and yeast and molds in tahini at 10^7, 10^2, and 10^3 CFU/g [27]. Similar studies and other official institutions have also set acceptable standards for tahini and ready-to-eat foods, as shown in Table III. According to Table III, the highest levels of acceptable microbial counts for APC, *S. aureus*, yeasts and molds, total coliforms, *E. coli* and *Salmonella* in CFU/g were as follows: 10^7 [27], 10^4 [28], $<10^6$ [28], 10^2 [28][29], <10 [26], <3 [29].

TABLE II. COMPARISION OF MICROBIAL RESULTS WITH LOCAL STANDARDS

Micro-organism	Unacceptable Limits	Unacceptable Samples N	% Unaccep-table
APC[a]	$1x10^4$CFU/g	29	46%
S. aureus[b]	$1x10^2$ CFU/g	33	52%
Yeast and molds[a]	$1x10^3$ CFU/g	42	67%
Total coliforms[a]	$1x10^2$ CFU/g	52	83%
E. coli[a]	10 CFU/g	23	37%
Salmonella[a]	0 CFU/g	10	16%

[a] obtained from LIBNOR standards [b] obtained from GSO standards N= Number of Samples

TABLE III. COMPARISION OF LIBNOR STANDARDS WITH INTERNATIONAL STANDARDS

	Microorganism CFU/g[a]					
Microbial Standards	***APC***	***S. aureus***	***Yeast/Molds***	***Coli-forms***	***E. coli***	***Salmo-nella***
LIBNOR [26]	10^4	-----	10^3	10^2	<10	0
FDA [30]	10^4	-----	10^3	10^2	-----	0
Boderck et al., (1990) [29]	$<10^5$	<20	------	$<10^2$	<3	<3
GSO [27]	10^7	10^2	10^3	------	0	0
Health Protection Agency [28]	10^6	10^4	$<10^6$	$<10^2$	0	0
Buyukunal, et al. (2010) [24]	10^5	10^2	10^4	460	9	0
New Zealand Ministry of health [31]	10^5	10^3	------	------	0	0

[a] CFU/g Colony forming units per gram

When compared to those standards, the APC, *S. aureus*, and yeast and mold counts for all the examined samples in this study are considered acceptable. However, many samples would still remain unacceptable with regards to total coliform counts, and the presence of *E. coli* and *Salmonella*. None of the tested samples should contain *Salmonella* due to its characteristic as a health hazard. According to GSO standards, none of the samples should contain even traces of *E. coli* [27]. The remaining samples should contain microbial levels below the acceptable limits in order to indicate good

implementation of manufacturing procedures as well as safe product manufacturing.

C. Impact Factors

Furthermore, the samples were grouped according to their respective manufacturer, processing method, and storage time, in order to identify certain impact factors.

Impact by manufacturer. Table IV shows the results obtained when the samples were grouped according to the manufacturer (also refer to figures 3 & 4). This is determined by ANOVA analysis, with significance indicated by a 95% confidence interval. Significant differences were seen for *S. aureus* counts, yeast and molds, and for the presence of *Salmonella*. For *S. aureus* counts, companies F and C showed significantly higher levels of average counts compared to other manufacturers. Manufacturer C also showed significantly higher levels of yeast and molds. Meanwhile manufacturers D, and G showed no signs of *Salmonella*, compared to other companies and differed significantly from company E, which showed the highest prevalence of *Salmonella* in the tested samples. No significant differences were detected for APC, coliform, and *E. coli* counts. Therefore there seems to be slight differences in microbial quality depending on the manufacturer, as well as the microbe under investigation.

These results are consistent with a similar study that determined significant differences between samples of tahini produced by different manufacturers [11]. The variations in microbial levels could be due to different manufacturing procedures and processing parameters. For example, the temperature and time of roasting for one manufacturer may be more efficient for reducing microbial levels than the time and temperature implemented by a different company. Also, the source of sesame seeds and water may differ according to manufacturer, which may also depend on the place of production. Another factor could be the temperature of the facility. Companies that are located in areas with elevated heights usually experience lower temperatures than companies located in coastal areas. Therefore, processing and storage conditions that favor the growth of microbes may lead to the variations in microbial levels, which could drastically increase in hot, humid areas.

TABLE IV. AVERAGE MICROBIAL COUNTS OF TAHINI SAMPLES GROUPED BY MANUFACTURER

	Microorganisms CFU/g					
Manu-facturer	*APC*	*S. aureus*	*Yeast/ Molds*	*Total Coli-forms*	*E. coli %*	*Salmo-nella %*
A	$1.9x10^{4a}$	$3.6x10^{2a}$	$1.2x10^{4a}$	$3.9x10^{4a}$	33^{a}	11^{ab}
B	$1.1x10^{5a}$	$2.7x10^{2a}$	$3.2x10^{4a}$	$3x10^{4a}$	11^{a}	22^{ab}
C	$1x10^{5a}$	$2.9x10^{3b}$	$7.3x10^{4b}$	$5x10^{4a}$	44^{a}	22^{ab}
D	$7.9x10^{4a}$	$2.1x10^{3a}$	$2.6x10^{3a}$	$1.9x10^{4a}$	44^{a}	0^{a}
E	$1.5x10^{5a}$	$6.9x10^{2a}$	$1.6x10^{4a}$	$1.7x10^{4a}$	33^{a}	44^{b}
F	$9.3x10^{4a}$	$1.3x10^{3ab}$	$2.7x10^{4a}$	$5.4x10^{3a}$	44^{a}	11^{ab}
G	$2x10^{4a}$	73^{a}	$1.3x10^{4a}$	$2.6x10^{3a}$	44^{a}	0^{a}

*Means within same row with different letters are significantly different ($p \leq 0.05$), CFU/g= Colony forming units per gram, Note: Nine samples were tested from each manufacturer

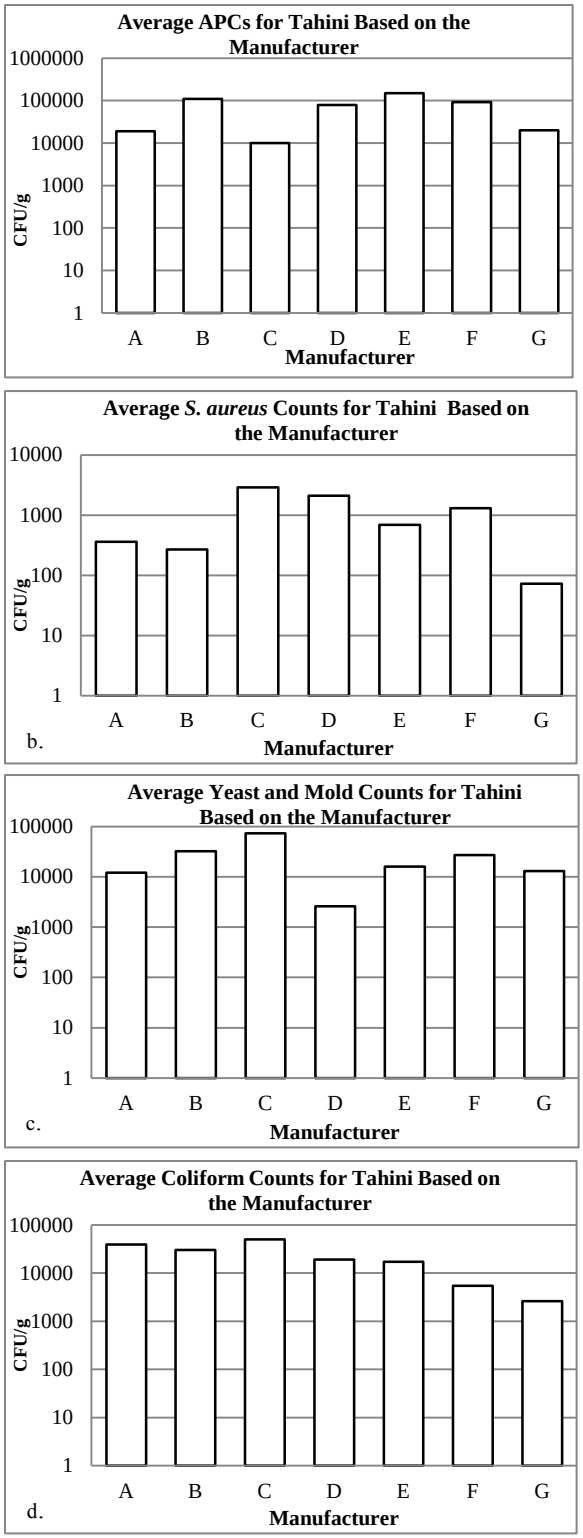


Figure 3. Average microbial counts in tahini samples according to the manufacturer (a) APC (b) *S. aureus* (c) Yeast/molds (d) Total coliform

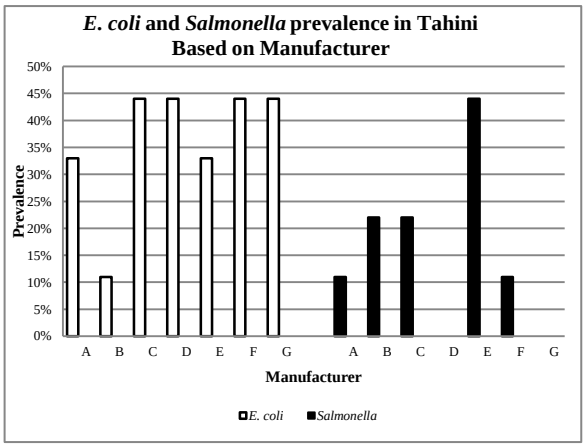


Figure 4. Prevalence of *E. coli* and *Salmonella* in tahini after grouping samples according to manufacturer

Another aspect may be the enforcement of HACCP plans and GMPs for production. Although the Lebanese government requires this, adherences to the regulations may be strictly enforced in one company yet loosely enforced in another. Whether or not the manufacturer produces tahini via the traditional or modern method may also play a role in determining levels of contamination as will be discussed.

Impact of Processing Method. The tahini samples were also grouped based on the processing method, i.e., whether the samples were produced via the traditional or modern method (solely automated machinery), and results were statistically interpreted based on a confidence interval of 95%. Significance was obtained via independent t-test analysis. Results are shown in Table V and Figures 5 & 6.

APCs were slightly higher for tahini produced by the modern method ($9.2x10^4$ CFU/g) in comparison to the average APC levels for tahini produced by the traditional method ($2x10^4$ CFU/g). The p-value for the differences was ≤ 0.05 and therefore it was significant. For average levels of *S. aureus*, tahini produced by the traditional method showed significantly lower counts (73 CFU/g) than tahini produced by the modern method ($9.6x10^2$ CFU/g). The p-value for *S. aureus* averages was also ≤ 0.05 and therefore the differences were significant. In the case of yeast and molds, the average for tahini produced by the modern method was slighter higher than the average for tahini produced by the traditional method. However, statistical analysis shows that this difference is not significant (Table V). Meanwhile, the average for total coliforms was $2.6x10^3$ CFU/g for traditionally produced tahini, and $2.7x10^4$ CFU/g for tahini produced by the modern method, with a p-value ≤ 0.05, which signals significant differences. Meanwhile, although there appeared to be a somewhat recognizable difference in *E. coli* levels, statistical analysis indicates that there is no significant difference between both groups for *E. coli* counts ($p>0.05$).

Additionally, no *Salmonella* was detected in tahini produced via traditional methods. All the samples containing *Salmonella* were produced by the modern method and this difference was also significant ($p\leq 0.05$). Hence, it appears that the manufacturing of tahini via the modern "machinery" method is contributing to higher microbial levels in the products.

In a similar study however, tahini samples produced by the traditional method came out as more contaminated than samples produced by the automated method with regards to average APCs, and *S. aureus* counts [11], however the study's results were consistent with our findings with regards to average counts for yeast and molds, which were higher for the modern manufacturing process.

TABLE V. AVERAGE MICROBIAL COUNTS OF TAHINI SAMPLES GROUPED BY PROCESSING METHOD

	Tahini Processing Method	
Microorganism CFU/g	*Traditional*	*Modern*
N	9	54
APC	$2x10^{4a}$	$9.2x10^{4b}$
S. aureus	73^a	$9.6x10^{2b}$
Yeast & molds	$1.3x10^{4a}$	$2.7x10^{4a}$
Total coliforms	$2.6x10^{3a}$	$2.7x10^{4b}$
E. coli %	44^a	35^a
Salmonella %	0^a	19^b

*Means within same row with different letters are significantly different ($p<0.05$), N= Number of samples, CFU/g=Colony forming unit per gram

It would have been expected that products obtained from traditional manufacturers contain higher levels of microbes, than products produced modernly due to a number of reasons including: lack of knowledge in HACCP principles, or the adherence to traditional production methods that may sometimes be unsanitary, as well as, the need to incorporate many staff members in small companies, which large manufacturing industries replace with machines. However, this was not the case in this study's findings. Therefore, the reason behind the high microbial levels is most likely due to the source of water used in production, as well as the roasting time. The traditional manufacturer usually roasts the seeds until confident that the seeds are free from contamination, usually determined by certain indicators, such as the color, smell, etc. Roasting in a modern industry, however, is done via a machine with fixated time and temperature, and whether or not all the seeds have been roasted to the sufficient level, they will move along on the processing line and any microbial growth will remain. Furthermore, seeds that are stored in large industries are packed together in large amounts. This atmosphere increases chances for microbial growth specifically, yeast and mold. Another factor could be difficulty in accessing guidelines for standards, safety procedures, and GMP policies, which are not readily available in Lebanon.

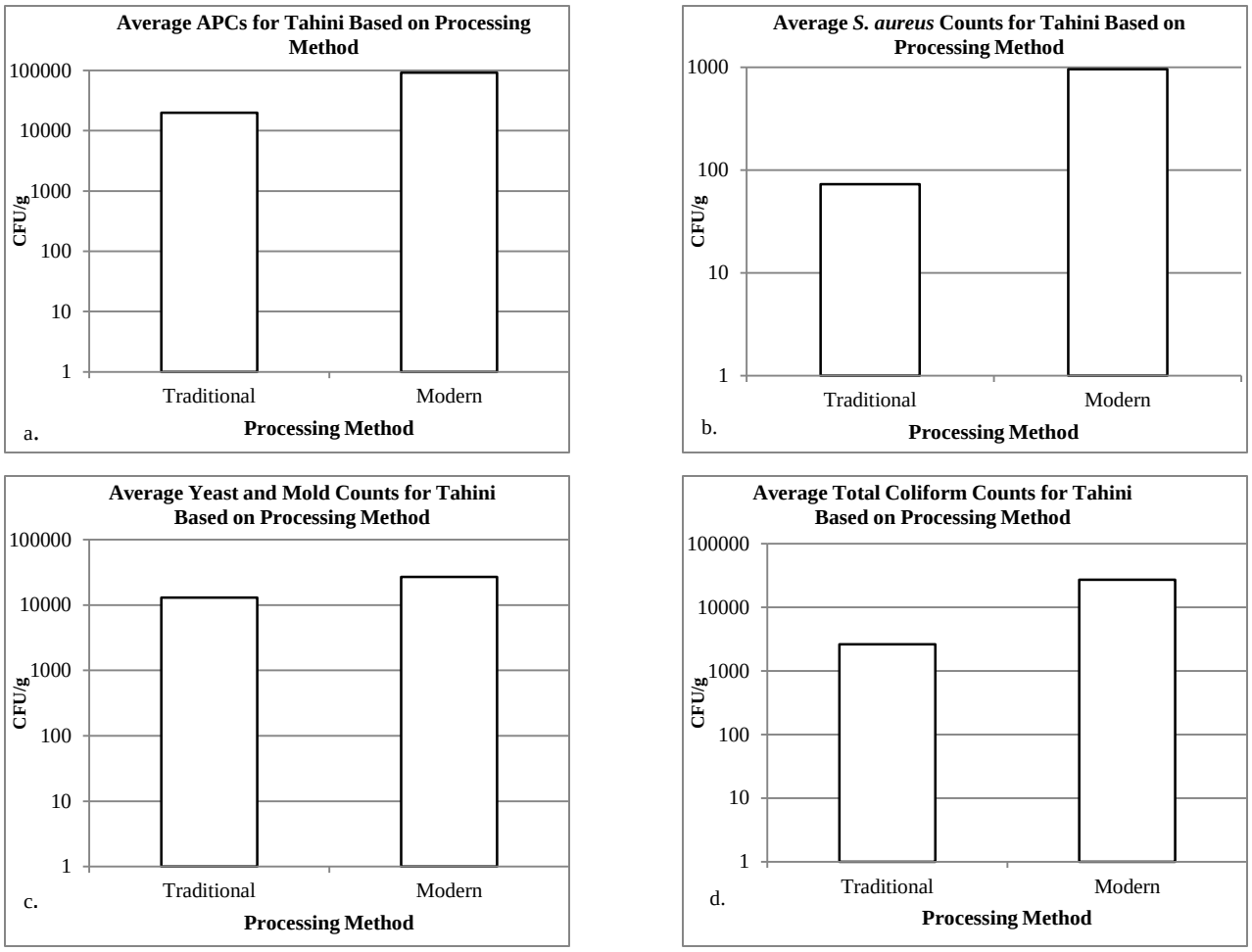


Figure 5. Average microbial counts for tahini based on processing method: (a) APC, (b) *S. aureus* , (c) Yeast and molds, (d) Total coliforms

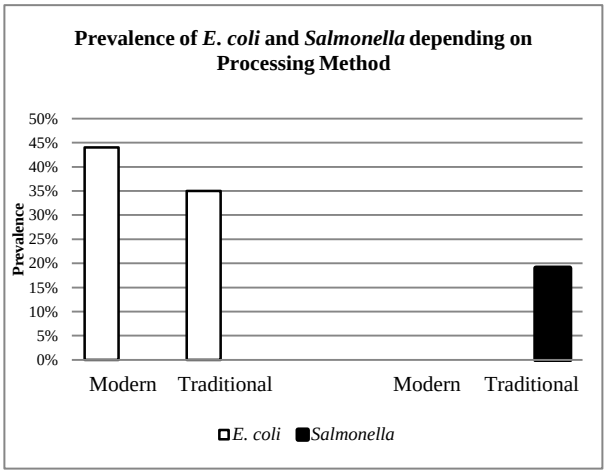


Figure 6. Prevalence of *E. coli* and *Salmonella* in tahini samples grouped according to sample age

Impact of Storage Time. The impact of the storage time was also assessed and the results of the statistical outcomes for the microbial counts are shown in Table VI (refer also to Figures 7&8). The samples were grouped based on whether the product was obtained directly from the manufacturer (fresh) or whether it spent a certain period of time (from 1 month to over 10 months) on the shelf. There were no significant differences among the samples with consideration for the sample age ($p>0.05$). Therefore the storage time seemed to have no significant impact on the microbial quality.

Although microbial counts were higher in products with a shelf life of over three months, products with shelf lives of over ten months showed sharp decreases in microbial quality. The fresh samples also appeared to have lower microbial qualities than older samples, yet similar to levels attained by samples over ten months.

In a similar study however, the microbial counts of tahini were seen to have significantly decreased after four months of storage [11]. The variations in our findings could be due to the fact it was not the same fresh sample but rather

samples from different batches that were examined, regardless of attribution to a same manufacturer.

TABLE VI. AVERAGE MICROBIAL COUNTS OF TAHINI SAMPLES GROUPED BY SAMPLE AGE

		Microorganisms CFU/g					
Sample age (Month/s)	*N*	APC	*S. aureus*	Yeast /Molds	Coli-forms	*E. coli* %	*Salmon -ella* %
Fresh	8	$1.8x10^4$	72	$1.1x10^4$	$2.8x10^3$	50	0
1	8	$2x10^4$	$1.4x10^3$	$1.5x10^4$	$4.5x10^4$	38	13
2	5	$1.4x10^4$	$4.1x10^2$	$1.6x10^4$	$4x10^3$	20	20
3	9	$4.3x10^4$	$1.3x10^3$	$3.1x10^4$	$4.5x10^4$	22	22
4	12	$1.5x10^5$	$7.3x10^2$	$3.7x10^4$	$3.1x10^4$	50	25
5	12	$1.6x10^5$	$1.4x10^3$	$3.9x10^4$	$1.2x10^4$	42	25
6-8	5	$1.3x10^5$	62	$7.9x10^3$	$2.4x10^4$	20	0
>10	4	$5.8x10^3$	$4.2x10^2$	$1.4x10^4$	$7.9x10^3$	25	0

Note: N=number of samples, CFU/g= Colony forming units per gram

Regardless however, it is evident that fresh samples and aged samples are vulnerable to microbial contamination. In addition, the results of the current study are uniform with results of similar studies that proved the existence of hazardous microbes in sesame paste products even after prolonged storage time.

Al-Holy et al. (2017) witnessed a decrease in *Salmonella* counts in halva with increased storage time [32]. The decrease in microbial levels could be due to bacterial competition for resources, which are limited in vacuum-sealed packages. However, even after a long period, the microbe was still present in hazardous levels, possibly due to the protective effect of high fat low moisture foods, for some bacteria [33][34]. Bacteria do not generally survive in dry environments, but some are able to survive in a dormant state, and when conditions are suitable once again, they are active, as is the case for *Salmonella*. Similar studies found that *Salmonella* survived in tahini even after 16 weeks of storage [35]. *Salmonella* was also seen to survive in tahini up to 8 months [36]. Meanwhile, Al-Holy et al. (2013) found that *E. coli* survived in tahini after 28 days of storage [37]. In halva, *S. aureus* was still present in the samples even after 9 months [23].

Tahini has a low water activity, and low water activity foods are considered safe from microbial contamination. However, this has not been the case. In the United States, 5,141 low-water activity food products were recalled from 2007 to 2012, due to bacterial pathogens [38]. Foodborne and waterborne pathogens can have deteriorative impacts on the quality of food, negative consequences to a person's health, and remain a major source for the spread of disease worldwide [40]. In general, contamination may occur from the use of contaminated water during washing, soaking, or brining, or cross contamination during processes that are open to air, for example, grinding or filling, or bad storage conditions within the factory [10].

On a more specific front, *S. aureus* often enter foods from the skin surfaces of employees [39]. The bacteria can be airborne and therefore exposure to the atmosphere for long periods of time can lead to high *S. aureus* counts. Major health implications can result from high microbial counts of *S. aureus*, which can also include toxin production [40].

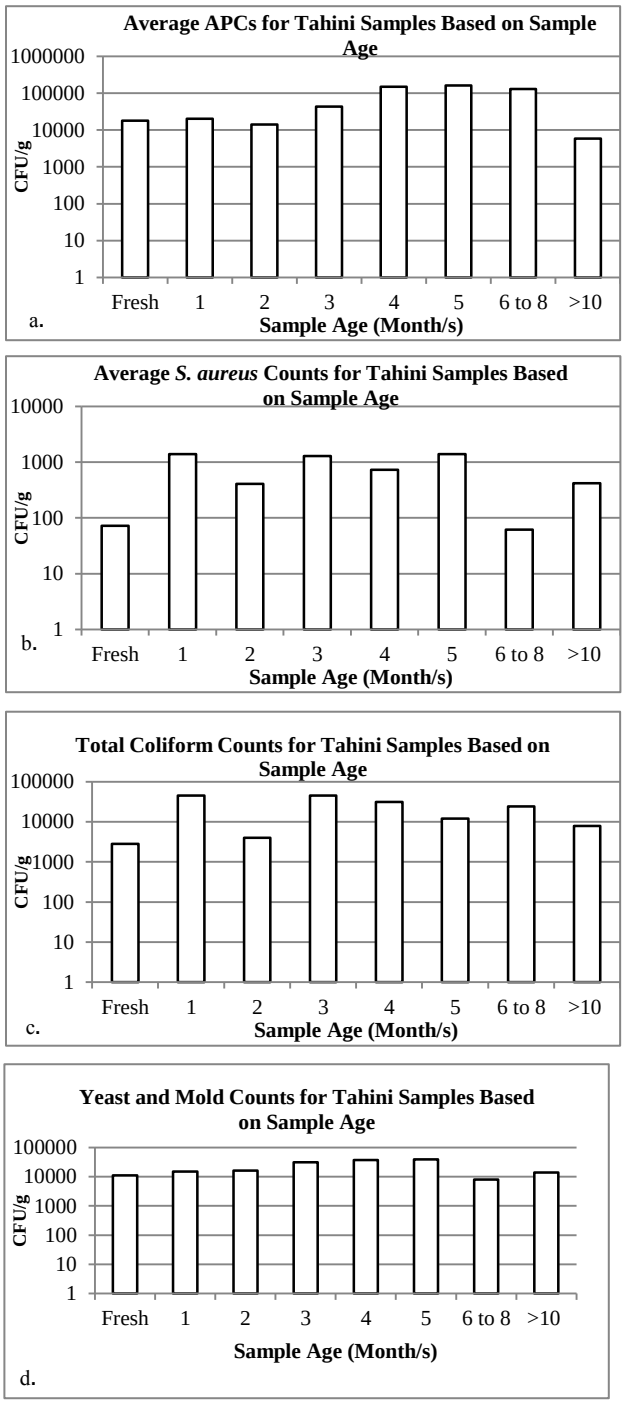


Figure 7. Average microbial counts in tahini samples grouped according to the sample age (a) APC, (b) *S. aureus*, (c) Yeast and molds, (d) Total coliforms

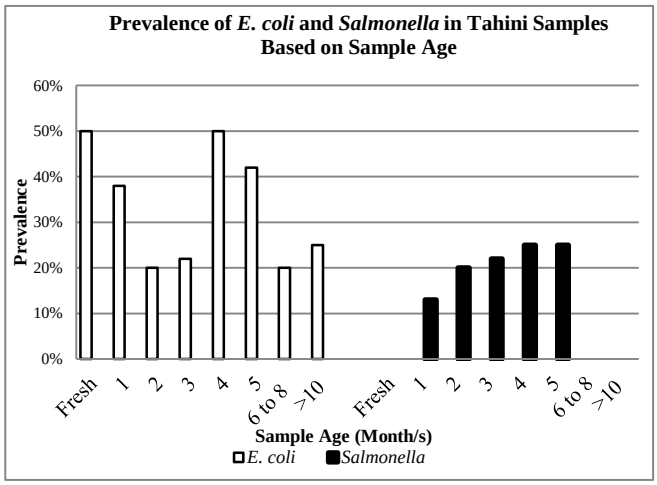


Figure 8. Prevalence of *E. coli* and *Salmonella* in tahini samples grouped according to sample age

Yeasts and molds are distributed widely in the environment as contaminants of air, water, soil and dust and present a potential threat due to mycotoxin production and allergic reactions [41]. During the study worms emerged on SDA agar, indicating the presence of insect eggs within the corresponding samples. Insect eggs can come as a result of improper handling and storage conditions and can also have negative implications to a person's health.

Coliforms on the other hand, principally *E. coli*, are widely used as indicators of human fecal contamination. Coliforms can cause gastrointestinal infections, and some strains of *E. coli* are also capable of causing serious kidney disease and can be fatal [39]. Presence of high coliform counts in food has been attributed to use of contaminated water during manufacturing [10]. Another microbe with fatal consequences upon ingestion is *Salmonella*. Various *Salmonella* species are commonly the etiological agents of salmonellosis, bacteremia, and typhoid fever, which require only minute amounts of *Salmonella* typhi [39]. The most commonly identified sources of *Salmonella* infections include birds and domestic fowl, including their eggs [39]. Contaminated water supplies and the handling of food products by individuals infected with this bacterium, could also lead to the spread of the microbe [10]. Insects, as well, are capable of transferring *Salmonella* upon ingestion or physical contact [42]. Inadequate roasting temperatures could lead to inadequate removal of *Salmonella* [10].

The obtained results indicate that some tahini produced in Lebanon is hazardous and could pose life-threatening consequences. In addition, about 35 percent of tahini produced in the country is exported, specifically to the USA, EU, Australia and the GCC. The products tested in this study include some of the country's major producers and exporters. The fact that Lebanese tahini has had incidents where *Salmonella* was detected, has reduced the quantities for export, especially to the USA [43]. Therefore the results indicate that Lebanese tahini could also have threatening consequences to health on a global scale, or to the country's profits from tahini exports.

This is the first collective study in Lebanon that determines the quality of tahini produced in the country by studying the microbial quality of the products via conventional plating methods, while also considering the sample age as well as the processing methods as possible impact factors. Although the paper does only consider Lebanese products, it is worth noting that all the studied samples are from companies that export tahini worldwide, making the problem a global concern. Also, other major worldwide exporters (e.g., Turkish, Jordanian, and Saudi Arabian companies) also carried out similar studies on the microbial quality of tahini and other sesame paste products, in their respective countries [9][11][19], and hence, this study is to complement the others. Furthermore the discovery of contaminated tahini products in other countries, (Turkey, Jordon, and Saudi Arabia) [9][11][19] motivated us to test the quality of tahini in Lebanon.

IV. Conclusion

The results of this study provided an evaluation into the microbial quality of sixty-three tahini products manufactured in Lebanon and showed that some products are unacceptable in accordance to local and international standards. The results were also determined to be somewhat influenced by the manufacturer, to which each sample belonged to, the processing method used for production, as well as the time the sample spent on the shelf.

Limitations include unequal sample sizes for the different factors studied (sample age, processing method) due to limited resources, and the randomization procedure.

Further testing will be required to determine the source of contamination in order to contain it. Furthermore, the study could be a basis for further research aimed to eradicate high microbial counts in tahini without impacting the overall physiochemical quality. A number of recommendations are advised for the government, industry, and consumer.

It is recommended that tahini manufacturers, as should be the case with all food manufacturers of ready-to-eat foods, adhere to and strictly enforce Good Manufacturing Procedures (GMPs), and HACCP procedures, and amend regulations if need be the case.

It is also recommended that further inspection of tahini, as well as other sesame seed products be tested for microbial stability to ensure safe manufacturing of products.

It is also advised that food manufacturing facilities implement HACCP plans set by the government, and when violations of such plans due occur, it is advised that severe consequences are imposed. Furthermore, strict regulations should ensure that exported tahini meets the regulations of the target countries to prevent recalls. It is suggested that government standards, HACCP plans, safety procedures, and other regulations be readily available to the manufacturer in order to encourage and ease implementation of GMPs.

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